CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

PIPERONYL BUTOXIDE

Chemical Code # 000486, Tolerance # 00127
SB 950 # 103

October 23, 1987
Revised: 10/12/89, 5/11/90, 9/24/91, and 11/27/95

I. DATA GAP STATUS

Chronic rat:	No data gap, no adverse effect
Chronic dog:	No data gap, no adverse effect
Oncogenicity rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect

Chromosome: No data gap, no adverse effect

DNA damage: No data gap, no adverse effect

Neurotox: Not required at this time

Note, Toxicology one-liners are attached

** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: T951127

Summary of Toxicology Data by C. Aldous, revised October, 1989 by J. Gee; May, 1990 by Aldous; Sept. 24, 1991 by T. Kellner, and 11/27/95 by Aldous and Gee.

All relevant records on file with the DPR Library as of 11/16/95 are included. This includes all record numbers up to 127126 (Document No. 127-062). Some older record numbers greater than 900000 are included.

These pages contain summaries only. Individual worksheets may contain additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED (CHRONIC AND ONCOGENICITY), RAT

NOTE: An NCI bioassay was published in 1979 (see under Rat Oncogenicity, 017:907618). In that study, which employed F-344 rats, there was an apparent dose-related increase in lymphomas, in females only. In that study, the incidence of lymphomas in concurrent control females was low, and incidence in concurrent male controls was unusually high. EPA determined, based on the unusual concurrent control incidences, that the study was not suitable to make scientific conclusions (see 013:907643, under Rat Oncogenicity, below). A replacement study was required. This was provided by the acceptable 1987 Bio-Research Laboratories study, immediately below. The latter study did not find increases in lymphomas in either sex of CD* rats. The overall conclusion of CDFA [now DPR] is that piperonyl butoxide is not oncogenic under conditions tested: this conclusion considers the limitations of evidence of a treatment effect (single sex in one study only), and recognizes that the more recent (negative) study meets more rigorous scientific standards of design and execution. Aldous, 5/10/90.

**127-028 074677 Graham, C., "24-month dietary toxicity and carcinogenicity study of piperonyl butoxide in the albino rat". Bio-Research Laboratories, Ltd., 8/27/87. Piperonyl butoxide, Batch Ref# FEG32, purity 89%, was administered in diet to Crl:CD* rats for two years. There were 120/sex in controls, and 60/sex in dosage groups of 30, 100, or 500 mg/kg/day. The effective NOAEL = 30 mg/kg/day for males and females [based largely on increased relative liver weights, associated with hepatocyte hypertrophy (500 mg/kg/day males and females) and increased incidence of focal eosinophilic cells (males) or focal mixed [staining] cells (females) at 100 and 500 mg/kg/day]. High dose groups had marked body weight decrements compared to controls (typically 100 g or more, both sexes). Some clinical chemistry effects reflected changes in liver function and consequential changes in general physiological status, particularly at high doses (elevated blood cholesterol, decreased blood glucose, decreased GOT and GPT levels). Thyroid changes included increased pigment in

colloid, and follicular hyperplasia (particularly at 500 mg/kg/day, both sexes). Incidence and degree of bilateral testicular atrophy appeared to be dose-related at 100 and 500 mg/kg/day. A minor increase in the degree of some normal degenerative changes in the ovaries was noted at 500 mg/kg/day. No adverse effects, considering the relatively high NOAEL. No oncogenicity effect was found. ACCEPTABLE. Aldous, 5/9/90.

127-028 086711 Test article analysis information relevant to study 127-028:074677, above, as well as to contemporary studies (rat reproduction study, 127-026:072948, and rabbit teratology study, 127-025:072947). All three studies used this same technical "composite", 89% purity, stable under test conditions. Data were considered in May, 1990 reviews. No CDFA worksheet. Aldous, 5/10/90.

CHRONIC TOXICITY, RAT

(Note acceptable "combined" rat study on previous page).

002 and 008 907616, "Chronic Oral Toxicity and Related Studies on Animals with the Insecticide and Pyrethrum Synergist, Piperonyl Butoxide - Rat Chronic, 2-Year Study." (U.S. industrial Chemicals Co. Research and Development Labs, 9/52) Technical piperonyl butoxide, 80%, was fed to Wistar rats in the diet at 0, 100, 1000, 10,000, or 25,000 ppm and 1000 ppm given in combination with 167 ppm pyrethrin, 12/sex/group, for 2 years. No apparent adverse effect: toxicity was limited to comparatively high doses. Apparent NOEL = 1000 ppm: dose-related decrease in body weight gain, and increase in degree and incidence of liver dysplasia and necrosis. Mortality was 100% at 2.5% in diet by week 78. UNACCEPTABLE and not upgradeable. Publication gives few details or histopathology, no individual data, no dose analysis presented, too few animals. (J. Remsen (Gee), 4/5/85).

002 038483 Brief summary of 907616.

CHRONIC TOXICITY, DOG

127-060 126286 Goldenthal, E.I., "Evaluation of Piperonyl Butoxide in a one year chronic dietary toxicity study in dogs", IRDC, Laboratory Project ID 542-005, 9/9/93. Piperonyl butoxide, purity of 90.78%, was administered in diets of 4 beagles/sex/group at 0, 100, 600 or 2000 ppm (corrected for purity) for one year. NOEL = 100 ppm [15% decrease in food consumption of males only). Findings in both sexes at 2000 ppm included decreased body weight, slightly decreased serum cholesterol and markedly increased alkaline phosphatase activity, increased liver weights, and mild diffuse hepatocellular hypertrophy (presumed related to the above changes). In addition, 2000 ppm males had 19% reduction in diet consumption and slight decreases in measured red blood cell parameters. Study is **acceptable. No adverse effects. Kishiyama and Aldous, 10/30/95.

127-063 129883 Errata submission for 127-060 126286, above. Changes do not affect acceptability of the report. Aldous, 10/30/95 (no worksheet).

127-059 126284 Goldenthal, E.I., "Evaluation of Piperonyl Butoxide in an eight week toxicity study in dogs", IRDC Laboratory Project ID 542-004, 9/9/93. This is the range-finding study to Record No. 126286. Piperonyl Butoxide, purity of 90.78%, was administered in diets of 2 beagles/sex/group at 0, 500, 1000, 2000, and 3000 ppm for 8 weeks. During the first week, all (and exclusively) 3000 ppm dogs showed inappetence and decreased defecation. Marked decrements in food consumption and body weight losses occurred at 3000 ppm: there were no definitive effects at lower dose levels. At 2000 to 3000 ppm, alkaline phosphatase was slightly elevated and cholesterol was slightly reduced, consistent with the chronic study. Liver weights appeared to indicate a dose-related increase over the entire dose range in males, but only at 2000 to 3000 ppm in females. Liver hypertrophy appeared to present a consistent trend across dose levels without a definite NOEL for males, however females indicated hypertrophy at 2000 and 3000 ppm only. This range-finding study does not indicate a "possible adverse effect". Small sample size makes this study of little value for "subchronic NOEL" determination. The dose levels selected for the chronic study are consistent with findings of this study. Aldous, 10/26/95.

002 and 008 38479 "Chronic Oral Toxicity and Related Studies on Animals with the Insecticide and Pyrethrum Synergist, Piperonyl Butoxide - Dog Chronic, 1-Year Study." (U.S. Industrial Chemicals Co. Research and Development Labs, 1952) Piperonyl butoxide, about 80%, given to mongrel dogs via capsule twice daily, 6 days/week for one year at 0, .003, 0.03, 0.1, or 0.3 ml/kg/day, 3/combined sexes/group. No apparent adverse effect: apparent NOEL = 0.03 ml/kg/day = 30 mg/kg/day (various tissue effects, including liver). UNACCEPTABLE and not upgradeable. No justification of dose, no analysis of dose, no individual data, too few animals, mongrels not acceptable. (J. Remsen (Gee), 4/5/85).

002 038482 Brief summary of 038479.

CHRONIC, (Other species)

002 and 008 038480, "Chronic Oral Toxicity and Related Studies on Animals with the Insecticide and Pyrethrum Synergist, Piperonyl Butoxide - Goat Chronic, 1-Year Study." (U.S. Industrial Chemicals Co. Research and Development Labs, 1952) One nanny goat was given a daily dose by capsule of 2.0 ml of piperonyl butoxide 6 days/week for 1 year. Goat and nursing kid were observed for effects. No apparent adverse effect: UNACCEPTABLE, not upgradeable. Not a guideline type study (one nanny goat and one kid are insufficient to evaluate chronic toxicity range). (J. Remsen (Gee), 4/5/85).

ONCOGENICITY, RAT

(See comments under "Combined, Rat", above.)

017 907618, "Bioassay of Piperonyl Butoxide for Possible Carcinogenicity - Rats." (NCI - sponsored study performed at Frederick Cancer Research Center, 8/7/78) Technical piperonyl butoxide, 88.4%, was fed to Fischer 344 rats in the diet for 107 weeks at 0, 5000 or 10,000 ppm. 50 rats/sex in treatment groups: 20/sex in controls. **Possible adverse effect is**

lymphomas in females. **Unacceptable, not upgradeable** (see also 013:907643, below). No analysis of dose, only two dose levels, insufficient numbers of controls, summary data only, no food consumption data. (J. Remsen (Gee), 4/8/85).

013 907643 Piperonyl Butoxide-Decision Document (EPA OPTS, 9/30/81). Discusses studies including 017:907618, above. The above NCI report concluded that piperonyl butoxide was not oncogenic in rats, since the incidence in high dose females was within historical control range, even though incidence was elevated compared to concurrent controls. EPA re-examined the data and determined that female lymphoma incidence was elevated compared to concurrent and to historical controls. Furthermore, the unusually low concurrent control incidence in females and unusually high incidence in concurrent control males "casts suspicion upon the whole study and, thus, no conclusions could be reached concerning piperonyl butoxide's carcinogenicity" (p. 2, executive summary, this record). EPA determined that a replacement rat oncogenicity study would be required (p. 11, this record). [One-liner only, no separate review of 013:907643.] (C. Aldous, 10/22/87).

002 and 003 031124 Less complete version of 907618.

ONCOGENICITY, MOUSE

****127-058 126278** Hermansky, S.J. and Wagner, C.L., "Chronic dietary oncogenicity study with Piperonyl Butoxide in CD-1* mice", Bushy Run Research Center, Export, PA, 8/27/93. Laboratory Project ID# 91N0134. Piperonyl butoxide, purity of 90.78%, was administered in diet at 30, 100, or 300 mg/kg/day to 60 D-1* mice/sex/group for at least 78 weeks. Two independent control groups, each with 60 mice/sex, were included in the study. No absolute NOEL was observed. A conservative NOAEL is 30 mg/kg/day, based on slight (not statistically significant) increases in liver weight in males at 30 mg/kg/day. No other effects were observed below 100 mg/kg/day. Hepatocellular changes were the primary findings: elevated incidence of hyperplasia and adenomas at 100 and 300 mg/kg/day in males and at 300 mg/kg/day in females. An increase in incidence of hepatocellular carcinomas in 300 mg/kg/day males,

although not statistically significant, was a plausible treatment effect. The study is **acceptable** as an oncogenicity study, with several deficiencies noted. The hepatocellular tumors constitute a **"possible adverse effect".** (Kishiyama and Aldous, 4/5/95).

127-047 118227 Preliminary report indicating increased masses in livers of male mice. (See final report, Record No. 126278, above).

127-056 120828 Preliminary histology data indicating increased tumors in livers of male and female mice. (See final report, Record No. 126278, above).

017 038485 "Bioassay of Piperonyl Butoxide for Possible Carcinogenicity-Mice." (Frederick Cancer Research Center, 8/7/78) Technical piperonyl butoxide, 88.4%, was fed to B6C3F1 mice in the diet at time-weighted average doses of 0, 1036 and 2804 ppm for 112 weeks, 50/sex /group, 20/sex/matched controls. No adverse effects indicated. UNACCEPTABLE, not upgradeable, (see also 013:907643, below). No analysis of dose, only two dose levels, too few concurrent controls, dose was changed during study, no individual data, no dates of deaths. (J. Remsen (Gee), 4/8/85).

013 907643 Piperonyl Butoxide-Decision document (EPA OPTS, 9/30/81). Discusses studies including 017:38485, above. EPA concluded that "further testing is necessary; the results of the NCI Bioassay are not definitive enough to judge whether PB [piperonyl butoxide] is positive or negative for carcinogenicity" (p. 11 of this record). EPA required a new mouse oncogenicity study. (C. Aldous, 10/22/87).

002 038484 and 002 and 003 031125 are summaries and partial duplicates of 038485. Evidently Record No. 907617 in this same volume refers to the same report (i.e., it bears the same title).

REPRODUCTION, RAT

**127-026 072948 Robinson, K. et al., "A Two-Generation (Two-Litter) Reproduction Study of Piperonyl Butoxide Administered in the Diet to the Rat", (Bio-Research Laboratories Ltd., Montreal, Quebec. Project no. 81689, July 1, 1986). Piperonyl Butoxide technical, lot FEG 32, 89% purity, was administered at concentrations of 0, 300, 1000, or 5000 ppm in the feed to 26 Sprague Dawley rats/sex/group for two generations, two mating periods/generation. Parental NOEL = 1000 ppm/day (reduced body weights in both sexes during growth phase). Developmental NOEL = 1000 ppm (reduced pup body weights; first apparent by about day 7 and progressively more marked by weaning at day 21). Reproductive NOEL = 5000 ppm/day (no adverse effects at the HDT). ACCEPTABLE, with no adverse effects. (Kishiyama, C. Aldous, 5/9/90).

002 and 008 038481, "Chronic Oral Toxicity and Related Studies on Animals with the Insecticide and Pyrethrum Synergist, Piperonyl Butoxide - Rat Reproduction Study." (U.S. Industrial Chemicals Co. Research and Development Labs, 1952). Technical piperonyl butoxide, 80%, was fed to Wistar rats in the diet for two years in a chronic study. During the study, animals were allowed to continually and randomly reproduce. Treatment levels were 0, 100, 1000, 10,000, or 25,000 ppm, 12/sex/group. No adverse effects indicated: apparent parental and reproductive NOELs = 1000 ppm; several reproductive indicators affected at 10000 ppm, which dose level markedly diminished weight gain in adults. No litters were produced at 25,000 ppm. Unacceptable, not upgradeable. Unacceptable protocol, too few animals, no analyses of doses, no records of matings. An adverse effect had been indicated in previous review [J. Remsen (Gee), 4/5/85]. The "adverse effects" classification was subsequently removed in view of substantial parental toxicity (C. Aldous, 10/22/87).

013 907643 Piperonyl Butoxide-decision Document (EPA OPTS, 9/30/81). Discusses studies including 002/008:38481, above. EPA concluded than an additional reproduction study was necessary (see p. 16 of this record).

TERATOLOGY, RAT

127-044 112421 Chun, J.S. and T.L. Neeper-Bradley, "Developmental toxicity evaluation of piperonyl butoxide administered by gavage to CD* (Sprague-Dawley) rats", Bushy Run Research Center, Export, PA., 12/20/91. Laboratory Project ID 54-586. "Piperonyl Butoxide FEP-100 Task Force Blend", purity 90.78%, was administered by gavage at concentrations of 0, 200, 500 or 1000 mg/kg/day to 25 mated Crl:CD* BR rats/group during gestation days 6 through 15. Maternal NOEL = 200 mg/kg/day (decreased body weights of dams). Common maternal findings at 1000 mg/kg/day included clinical observations of urogenital area wetness, and minor increases in liver weights. Developmental NOEL = 200 mg/kg/day (equivocal minor decrement in ossification of cervical centra #5 and #6). Study is **acceptable, with **no adverse effects**. Kishiyama and Aldous, 11/15/95.

127-050 119649 Chun, J.S. and T.L. Neeper-Bradley. Range-finding study to primary study 127-044 112421, above. Dose levels of 250, 500, 1000, 2000, or 4000 mg/kg/day were administered on gestation days 6-15 to groups of 5 Crl:CD* BR rats/group. Rats were observed until scheduled sacrifice on day 21 p.c. Gestational parameters were recorded, and fetuses were given external examinations and discarded. All high dose dams and 4/5 of the 2000 mg/kg/day dams died or were killed *in extremis*. Ulceration of the glandular stomach and sloughing or hemorrhaging of the non-glandular stomach were commonly observed in these dams. Urogenital area wetness was observed in 3/5 1000 mg/kg/day dams. Body weight gain was reduced in 500 and 1000 mg/kg/day dams over the period of days 12-15 p.c. There were no identified developmental effects in the survivable dose range (up to 1000 mg/kg/day). This study supports the dose levels selected for the primary teratology study, above. **No adverse effects are indicated**. No worksheet is necessary. Aldous, 11/15/95.

002 021597, "Teratogenic Study with Piperonyl Butoxide Technical in Albino Rats." (IBT, 6/10/76) Piperonyl butoxide was given to rats to gavage on days 6 to 15 of gestation at 0 (Corn oil), 300 or 1000 mg/kg, 19/sex/group. No effects reported. Invalid IBT study. UNACCEPTABLE, not upgradeable. (J. Remsen (Gee), 4/8/85).
EPA one-liner: Invalid.

TERATOLOGY, RABBIT

**025 072947 Leng, J. M., et al., " Teratology study in rabbits", IRDC, 2/7/86. Piperonyl Butoxide, purity 89%, administered by gavage at 0 (Mazola* corn oil), 50, 100, or 200 mg/kg/day to 16 female New Zealand White rabbits per group during gestation days 7 through 19. Maternal NOEL = 50 mg/kg/day (decreased quantity of stool: body weight losses). Developmental effects NOEL = 200 mg/kg/day (no apparent treatment effects). No adverse effects. ACCEPTABLE. (Kishiyama, C. Aldous; 5/9/90).

GENE MUTATION

020 034982, 034983, 068746 "Evaluation of Piperonyl Butoxide in the CHO/HGPRT Mutation Assay With and Without Metabolic Activation", (Arthur D. Little Inc., 5/1/85, amended report 2/12/86). Piperonyl butoxide (PB), lot FEG-32, technical grade, was used in the CHO/HGPRT assay. CHO-K_1 BH_4, tested without activation at the concentrations of 0 (medium and DMSO), 10, 25, 50, 75 and 100 µg/ml, 16 hour incubation; with Aroclor-induced rat liver activation (final protein concentration of 1-2 mg/ml), 0 (medium and S9), 25, 50, 100, 250 and 500 µg/ml, 5 hour incubation. Duplicate cultures, 5 plates each, equivocal results complicated by precipitation problems. No apparent adverse effects. Initially evaluated as unacceptable, not upgradeable - no repeat experiment. (J. Remsen (Gee), 10/30/85). Amended final report (068746) includes some additional data on the original trial and a letter from Arthur D. Little, dated 2/29/88, which agrees the test should be repeated. Status of study remains UNACCEPTABLE, not upgradeable. (Kishiyama/J. Gee, 10/11/89).

020 068746. A more complete report of 034984.

020 034983. Addendum to 034982.

003 907619 "NTP Technical Bulletin-Mutagenesis Testing Results - Salmonella typhimurium." (SRI International, 12/17/80) Piperonyl butoxide, no purity given, was assayed with S.

typhimurium strains TA100, TA98, TA1537 and TA1535 at concentrations of 0, 100, 333, 1000, 3333, or 10,000 µg/plate, with and without rat and hamster liver activation, 3 plates/concentration, two trials using preincubation in suspension. UNACCEPTABLE, possibly upgradeable with submission of full report. No positive controls, precipitation of test compound with increasing concentration and toxicity as seen by "partial clearing of background lawn." No apparent adverse effects: (one test with TA1535 is noted as positive, but is questionable with no dose response at 0 to 2000 ug/plate in second trial). (J. Remsen (Gee), 4/8/85).

** 127-037 98049 Lawlor, T. "Mutagenicity Test on Piperonyl Butoxide in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test) with a Confirmatory Assay" (Hazleton Washington, Inc., HWA #14413-0-401R, 7/29/91). Piperonyl Butoxide, FEP-100 Task Force II Blend, 90.78% A.I. was tested with Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538, with and without metabolic activation by Aroclor 1254-stimulated rat liver S-9 fraction with 3 plates/strain/dose in 2 trials (plus repeat of experiment with strain TA1537); dose levels were 100, 333, 667, 1000, 3330, 5000 ug/plate. No adverse effects were noted (no increase in the number of revertant colonies); ACCEPTABLE. (Kellner and Gee, 9/10/91).

127-038 98290 is a duplicate of -037:98049 with the following amendments: Appendix A and B were paginated; paging range and final report date on p. 1 were changed.

CHROMOSOME EFFECTS

** 127-038 98289 Murli, H. "Mutagenicity Test on Piperonyl Butoxide Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Multiple Harvests under Conditions of Metabolic Activation" (Hazleton Washington, Inc., HWA #14413-0-437C, 8/28/91). Piperonyl Butoxide, FEP-100 Task Force II Blend, 90.78% A.I. was tested for chromosome aberration potential in Chinese hamster ovary (CHO) cells with and without metabolic activation by Aroclor 1254-stimulated rat liver S-9 fraction with 2 cultures/dose in 2 trials (10 and 20 hour treatments). Dose levels were 0 (controls), 25.0, 49.9, 74.9 and 99.9 ug/ml without S-9 and 0, 62.6, 125, 188 and 251 ug/ml with S-9. No adverse effects were noted (no increase in the proportion of aberrant metaphases at any dose level). ACCEPTABLE. (Kellner and Gee, 9/13/91).

127-037 98050 is a duplicate of -038:98289, except for the following items that are missing in 98050: pages 2, 3, 10 and 22 and pagination in Appendix II. Also, paging range and final report date on p. 1 were amended in 98289.

** 062 127176, "Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Multiple Harvests under Conditions of Metabolic Activation with Piperonyl Butoxide", (H. Murli, Hazleton Washington Inc., HWA 14413-1-437C, 9/20/93). Chinese hamster ovary cells were evaluated for chromosomal aberrations after exposure to piperonyl butoxide, 90.8% ai, at concentrations of 0, (DMSO) 15, and 20 µg/ml, without S9 or 12, 30, 60, 90, and 120 µg/ml with S9 at the 10 hour harvest and in a second test at 20, 25, and 30 µg/ml without S9 or at 30, 60, 90, 120 µg/ml with S9 at 20 hours (plus a 30 hour harvest for the high dose only) after treatment. Decreases in the numbers of mitotic cells were observed, except at the low dose, with increased harvest time. The numbers of cells with chromosome aberrations did not increase significantly with piperonyl butoxide under the conditions of this study. ACCEPTABLE. (Kishiyama and Gee, 11/16/95)

DNA DAMAGE

** 127-037 98051 McKeon, M. "Genotoxicity Test on Piperonyl Butoxide In The Assay For Unscheduled DNA Synthesis In Rat Liver Primary Cell Cultures With A Confirmatory Assay" (Hazleton Washington, Inc., HWA #14413-0-447R, 7/29/91). Piperonyl Butoxide, FEP-100 Task Force II Blend, 90.78% A.I. was tested for unscheduled DNA synthesis (UDS) potential in vitro using cultured cells from male Fischer 344 rat liver with 3 cultures/dose at 0 (DMSO), 1.00, 2.50, 5.00, 10.0, 25.0, 50.0 and 100 ug/ml in Trial 1 and 0 (DMSO), 2.50, 4.99, 9.98, 25.0, 37.4, 49.9 and 74.9 ug/ml in Trial 2 (top doses in each trial were not scored because of cytotoxicity). No adverse effects were noted (i.e. piperonyl butoxide did not induce DNA damage in cultured rat liver cells at the doses tested). ACCEPTABLE. (Kellner and Gee, 9/17/91).

127-038 98291 is a duplicate of -037:98051 with the following amendments: Appendix B and C were paginated; paging range and final report date on p. 1 were changed.

NEUROTOXICITY

Not required at this time.